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Pre-clinical evaluation of combinations of PI3K & MEK inhibitors in colorectal carcinoma cell lines

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MAPK and PI3K signalling pathways are frequently activated in human cancers, and represent promising therapeutic targets. Previous studies suggest that combined targeting of these pathways may be necessary for optimal therapeutic activity, hence the aim of this study was to evaluate the MEK inhibitors, ARRY-142886 and PD 0325901, alone and in combination with the dual mTOR/PI3K inhibitor, NVP-BEZ235, or the pan class I PI3K inhibitor, GDC-0941, in colorectal cancer cell lines. Growth inhibition, survival and signal transduction were measured using the Sulforhodamine B assay, clonogenicity and western blotting, respectively. Median effect analysis revealed that all MEK/PI3K inhibitor combinations exhibited marked synergistic growth inhibition in both HCT116 and HT29 cell lines. GDC-0941 displayed the greatest synergy in combination with either MEK inhibitor. At concentrations up to 10 μM only NVP-BEZ235 was cytotoxic after 72 hours exposure in either colorectal cancer cell line with an LC_{50} of 0.51 μM in the HCT116 cell line and an LC_{50} of 0.49 μM in the HT29 cell line, and increased cytotoxicity was only observed with selected MEK/PI3K inhibitor combinations in the HT29 cell line. Western blotting revealed that NVP-BEZ235 exhibits stronger inhibition of 4EBP1 phosphorylation, and similar inhibition of S6 and AKT phosphorylation, compared to GDC-0941. Both PD0325901 and ARRY-142886 inhibited ERK phosphorylation. The additional effect on S6, ERK or AKT phosphorylation observed with MEK/PI3K inhibitor combinations was minimal. However, there is a significant difference in the magnitude of inhibition of p4EBP1, as with NVP-BEZ235 there is a high level of inhibition alone and in combination whereas there is minimal inhibition with GDC-0941. These studies confirm that single agent MEK and PI3K inhibitors are predominantly cytostatic, as opposed to cytotoxic; however, combination studies demonstrated marked synergism in the 2 cell lines investigated. The dual mTOR/PI3K inhibitory action of NVP-BEZ235 may increase its ability to inhibit 4EBP1 phosphorylation, and thereby protein translation. Furthermore, the lower level of synergy exhibited by NVP-BEZ235 in combination with MEK inhibitors, compared to GDC-0941, may be due to the inhibition of mTOR and thereby 4EBP1 phosphorylation. These studies confirm that dual targeting of PI3K and MEK can induce synergistic growth inhibition; however, the detailed effects of specific inhibitors should be investigated to identify optimal combinations.
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Evaluation of the synthetic heat shock protein 90 inhibitors NVP-AUY922 and NVP-HSP990 in human prostate cancer tissue

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The molecular chaperone heat shock protein 90 (Hsp90) is an important target for cancer therapy as it is required for the correct maturation and function of its various client proteins, many of which are known oncogenes. In prostate cancer, targeting Hsp90 is particularly attractive as the androgen receptor (AR), the key mediator of prostate cancer cell growth and survival, is also an Hsp90 client protein. Despite promising results in pre-clinical studies, the first-in-class Hsp90 inhibitor 17-allylamino-demethoxygeldanamycin (17-AAG) has shown limited efficacy in phase I and II clinical trials for advanced prostate cancer, at least in part due to limitations in formulation, poor pharmacokinetics and hepatoxicity. In this study, we used cell based assays and a model of human prostate cancer to examine the efficacy of two new synthetic inhibitors of Hsp90, namely (i) NVP-AUY922 that has emerged as the most potent Hsp90 inhibitor developed to date, and (ii) the orally available NVP-HSP990. We demonstrate that both agents are significantly more potent than 17-AAG at killing prostate cancer cells. In the AR-positive LNCaP cell line, a 40 nM dose of NVP-AUY922 or NVP-HSP990 induced cell death in 70% and 30% of cells, respectively, compared to no cell death observed with 40 nM 17-AAG. The AR-negative cell line PC3 was more sensitive to both agents, with a 40 nM dose of NVP-AUY922 or NVP-HSP990 causing 80−90% cell death. Both NVP-AUY922 and NVP-HSP990 significantly reduce steady-state protein levels of the Hsp90 client proteins HER2, c-RAF-1 and AR, in addition to the AR-regulated protein PSA, and both inhibitors altered cell cycle distribution. In addition to our cell line studies, we have developed a unique model of human prostate cancer where specimens collected from men undergoing radical prostatectomy are cultured as explants. Using this model, we demonstrate for the first time how human prostate tumour tissue responds to NVP-AUY922 and NVP-HSP990, and demonstrate modulation of the established clinical biomarkers of Hsp90 inhibition, namely HSP70, c-RAF-1 and CDK4, in addition to AR. In summary, we provide the first extensive evaluation of the synthetic Hsp90 inhibitors NVP-AUY922 and NVP-HSP990 in prostate cancer cells and human prostate cancer tissue. Our studies support clinical development of these agents for the treatment of prostate cancer.

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Pre-clinical evaluation of LYS6KAKT1, a novel, highly selective, orally bioavailable dual inhibitor of p70 S6 Kinase and AKT currently in phase I clinical trials for cancer

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PI3K/AKT/mTOR/S6K signaling pathway (AKT pathway) controls cell survival, cell-cycle progression, cell growth and metabolism through a cascade phosphorylation of a number of key substrates. This pathway is regulated by three well characterized tumor suppressors; *pten, tsc2,* and *lkb1*. Deletion of these genes results in activation of the AKT pathway and proliferative disorders. Similarly, activating mutations of the receptor tyrosine kinases or PI3 Kinase result in the activation of the pathway. Therefore, multiple nodes of the pathway have become drug targets. As part of a comprehensive drug discovery platform aimed at targeting the PI3K pathway, we have developed a potent small molecule dual inhibitor of p70 S6 kinase and AKT targeting two key nodes of the pathway.
LYS6KAKT1 is a potent, highly selective ATP competitive inhibitor against p70 S6 kinase and AKT with an IC50 of 6 nM and 14 nM respectively. *In vitro*, LYS6KAKT1 inhibits the phosphorylation of S6 ribosomal protein in U87MG glioblastoma cells with an IC50 of 120 nM and the phosphorylation of GSK3b with an IC50 of 1200 nM. It also inhibits the phosphorylation of other downstream AKT substrates such as PRAS40 and FOXO. Similar activity is seen in a broad range of other cell lines. *In vivo,* LYS6KAKT1 demonstrates potent phospho-S6 inhibition in nude mice bearing U87MG glioblastoma cells, with an ED50 value of 3 mg/kg and an ED90 value of 8 mg/kg 4 hours after a single oral dose. *In vivo* pharmacodynamic activity on phospho-S6 was demonstrated in other xenograft models such as A2780 (ovarian), H460 (lung), PC3 (prostate), and HCT116 (colon). In addition, pharmacodynamic activity was shown on other AKT markers such as phospho-PRAS, phospho-Forkhead, and phospho-S6K. In these studies, LYS6KAKT1 showed elevation of phospho-AKT in a dose dependent manner. This has been observed with other ATP competitive AKT inhibitors, whereas a previously disclosed p70 S6 kinase selective inhibitor LYS6K1, did not (Geeganage et al, Abstract 352, Pre-clinical evaluation of LYS6K1, a novel, highly selective, orally bioavailable inhibitor of p70 S6 Kinase currently in phase I clinical trials for cancer, AACR 2010). In vivo pharmacodynamic relationships on pS6 and other markers were dose, exposure, and time dependent.
In vitro, LYS6KAKT1 also showed cellular anti-proliferative activity in a broad range of cell lines in monolayer and colony formation assays. *In vivo,* LYS6KAKT1 effectively inhibits the growth of A2780 ovarian carcinoma xenografts in mice and the growth of U87MG glioblastoma tumor as a single agent at 8 mg/kg and 2.5 mg/kg given twice daily, respectively. It also inhibits the growth of 786-O renal xenograft growth when given orally once a day at 12.5 mg/kg.
Based on above pre-clinical observations, LYS6KAKT1 is currently being evaluated in phase I clinical studies for cancer.

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Efficacy evaluation of novel Pim kinase inhibitors with anticancer activity

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Pim-1, -2, and -3 serine-threonine kinases play an important role in intracellular signaling and contribute to pathways involved in cell survival, proliferation, stress response and cellular motility. Pim kinases emerged as a novel and interesting target of significant potential for therapeutic intervention in cancer. Overexpression of Pim kinases was reported for a variety of cancer types of both hematological and solid tumor type origin such as diffuse B cell lymphoma, chronic lymphocytic leukemia, Flt3-mediated acute myelogenous leukemia, prostate, pancreatic and hepatic cancers.
Selvita is presenting results of the currently performing lead optimization program of novel, small molecule Pim kinase inhibitors. Among the newly synthesized compounds we have identified molecules exerting substantial specificity and superior potency in inhibition of all three Pim kinase isoforms